

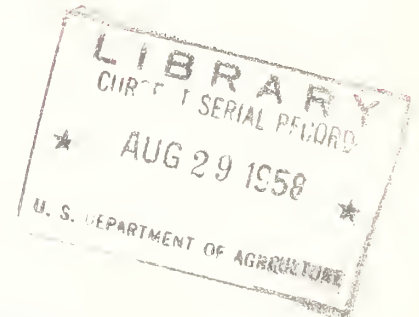
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United States Department of Agriculture
Agricultural Research Service



Report
of

ANNUAL CORN AND WHEAT UTILIZATION CONFERENCE

Northern Utilization Research and Development Division
and
Corn Industries Research Foundation
Technical Committee

Peoria, Illinois
June 3, 1958

TABLE OF CONTENTS

	<u>Page</u>
I. Attendance List	1
II. Program	4
III. Introduction	5
IV. Research Recommendations of the Technical Advisory Committee	7
V. Progress Reports on Selected Projects at NU and Discussions on These Reports	10
A. Effect of Granule Pretreatment on the Intrinsic Viscosity of Amylose in Aqueous Extracts of Corn and Wheat Starches (E. M. Montgomery and F. R. Senti)	10
B. Nonprotein Nitrogenous Constituents of Corn (J. S. Wall)	10
C. Composition of Cementing Layer Between the Germ and Endosperm in Corn (M. J. Wolf)	11
D. Effect of Drying Conditions on the Suitability of Artificially Dried Corn for Starch Production (M. M. MacMasters)	12
E. Dialdehyde Starch Production -- Demonstration of Electrolytic Cells in Pilot Plant (V. E. Sohns)	13
F. Fractionation of Corn Protein (E. M. Craine)	14
G. Microbial Production of β -Carotene (Alex Ciegler)	15
H. Cooperative Program on the Development of High- Amylose Corn (F. R. Senti)	16
VI. Closing Remarks	19

I. LIST OF ATTENDANCE

CORN INDUSTRIES RESEARCH FOUNDATION TECHNICAL COMMITTEE

Corn Industries Research Foundation, Inc., Washington, D. C.

Dr. J. T. Goodwin, Jr., Technical Director
Mr. F. J. Hosking, Executive Vice President

American Maize-Products Company, Roby, Indiana

Dr. J. W. Evans, Director of Research

Anheuser-Busch, Inc., St. Louis, Missouri

Dr. R. J. Sumner, Director, Central Research Department
Dr. B. L. Scallet, Associate Director, Central Research Department
Dr. A. J. DeGrand, Technical Manager

Clinton Corn Processing Company, Clinton, Iowa

Mr. G. T. Peckham, Jr., Research Director
Dr. J. M. Newton, Director, Technical Sales Service

Corn Products Refining Company

Mr. R. G. Ruark, Vice President (New York, New York)
Dr. A. L. Elder, Director of Research (Argo, Illinois)
Dr. A. L. Wilson, Assistant Director of Research (Argo, Illinois)

The Hubinger Company, Keokuk, Iowa

Mr. John Harness, Chief Chemist
Mr. Lee Elizer, Research Chemist

National Starch Products, Inc., Plainfield, New Jersey

Dr. C. G. Caldwell, Vice President - Research

Penick and Ford, Ltd., Inc., Cedar Rapids, Iowa

Mr. W. C. Black, Research and Development
Mr. E. T. Hjermstad, Research Chemist

A. E. Staley Manufacturing Company, Decatur, Illinois

Dr. T. L. Gresham, Vice President
Dr. J. A. Bralley, Director of Research

Union Starch and Refining Company, Granite City, Illinois

Dr. R. E. Pyle, Research Director
Dr. J. F. Lenney, Research Chemist

NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

Dr. W. D. MacLay, Director
Mr. C. E. Rist, Assistant Director
Mr. L. L. McKinney, Assistant Director
Mr. D. H. Mayberry, Information Specialist

Cereal Crops Laboratory

Dr. F. R. Senti, Chief
Mr. D. D. Christianson
Dr. E. M. Craine
Mr. W. L. Deatherage
Dr. R. J. Dimler
Mr. H. L. Griffin
Dr. L. A. Gugliemelli
Mr. J. E. Hodge
Mr. B. T. Hofreiter
Dr. Samuel Levine
Mr. E. D. Lukasik
Dr. M. M. MacMasters
Dr. C. L. Mehltrittter
Dr. R. L. Mellies
Miss E. M. Montgomery
Mr. J. C. Rankin
Dr. C. R. Russell
Mr. W. C. Schaefer
Dr. S. M. Schwartz
Mr. H. L. Seckinger
Mr. J. W. Sloan
Mr. H. E. Smith
Dr. N. W. Taylor
Mr. Robert Tobin
Dr. J. W. Van Cleve
Dr. J. S. Wall
Dr. M. J. Wolf
Mr. H. F. Zobel

Engineering and Development Laboratory

Mr. E. L. Griffin, Jr., Chief
Mr. Roy A. Anderson
Mr. Charles Vojnovich
Mr. V. E. Sohns

Fermentation Laboratory

Dr. R. W. Jackson, Chief
Dr. Ralph F. Anderson
Dr. Alex Ciegler
Dr. H. H. Hall

Forage and Industrial Crops Laboratory

Dr. I. A. Wolff, Chief
Dr. C. R. Smith, Jr.

Oilseed Crops Laboratory

Dr. F. C. Mayer
Dr. J. J. Rackis
Dr. A. K. Smith
Dr. W. J. Wolf

II. United States Department of Agriculture
Agricultural Research Service

PROGRAM

ANNUAL CORN AND WHEAT UTILIZATION CONFERENCE

Northern Utilization Research and Development Division
and
Corn Industries Research Foundation
Technical Committee

Peoria, Illinois
June 3, 1958

Morning Session - 9:30 a.m.

Introduction W. D. Maclay
Effect of Granule Pretreatment on the Viscosity
of Amylose in Aqueous Starch Extracts E. M. Montgomery
Nonprotein Nitrogenous Constituents of Corn J. S. Wall
Research Recommendations of the Technical Advisory Committee

Lunch - 12:30 p.m.

Afternoon Session - 2:00 p.m.

Composition of Cementing Layer Between the Germ
and Endosperm in Corn M. J. Wolf
Effect of Drying Conditions on the Suitability of
Artificially Dried Corn for Starch Production M. M. MacMasters
Dialdehyde Starch Production--Demonstration
of Electrolytic Cells in Pilot Plant V. E. Sohns
Fractionation of Corn Protein E. M. Craine
Microbiological Production of β -Carotene Alex Ciegler
Cooperative Program on the Development of
High-Amylose Corn F. R. Senti

Closing Remarks

III. INTRODUCTION W. D. Maclay

Dr. Maclay extended a cordial welcome to members of the CIRF Technical Committee and guests at the Sixth Annual Meeting of the current series, and stated that we had found information and suggestions coming out of these meetings most helpful in the development of our research program on corn and wheat. This meeting, he said, promised to be even more fruitful inasmuch as the Technical Committee was for the first time presenting, as a separate part of the program, their views on some needs in the field of corn research.

Changes which have been made in our organization during the past year were reviewed. This included mention of the six laboratories (Cereal Crops, Oilseed Crops, Fermentation, Forage and Industrial Crops, Engineering and Development, and Pioneering) within the Division and a brief discussion of their areas of responsibility. Under the new setup, it was pointed out that the Director's Office was being expanded to relieve the Laboratory Chiefs of the administrative burden thus enabling them to devote more time to research.

Directing attention to the research program of the Northern Division, Dr. Maclay stated that the number of line projects had been reduced considerably to permit more emphasis to be placed on areas which showed greatest promise of leading to early developments of major significance to utilization. In this connection it was pointed out that because of the promising outlook for fatty alcohol vinyl ethers as coatings for metals, the industrial potentialities of dialdehyde starch, and the foreseeable development of agronomically and industrially suitable high-amylose corn, teams of from 20-30 workers had been assigned to work in each of these areas of research and development. Dr. Maclay stated that the Forage and Industrial Crops Laboratory was engaged in a program of screening plants from all over the world in an effort to find potential new crops having the following characteristics: (1) High protein content with improved amino acid composition; (2) high oil content with new fatty acids or comprised mainly of a single fatty acid; (3) polysaccharide gums, nonstarch-like in character and readily obtainable in high yields; (4) high-fiber content, high yields per acre, and easy to process for fiber; and (5) valuable minor constituents such as enzymes, nutritional factors, pharmaceuticals, etc. Thus far slightly over 2,000 accessions of plant material have been received and catalogued. Nearly 1,000 of these accessions have been analyzed for seed and fiber composition. Of 621 seed samples analyzed for oil and protein content, 37 have been found with protein content as high or higher than soybeans, and 106 had an oil content equal to or greater than soybeans. Unusual amounts and types of unsaturation were found in several of these oils. Of the 87 oil samples analyzed, 16 were found to contain epoxy acids, 10 contained hydroxy acids, and 13 contained more than 10 percent of acids other than the usual C_{16} and C_{18} acids.

The completion of a literature survey on the amino acid content of seeds from a wide variety of plant families and species was also cited as

another significant accomplishment. This survey showed that the seeds of many plants contain lysine, methionine or tryptophan in amounts well above the average for corn and soybeans thus pointing up the possibility of finding or developing new and unusual crops.

Among a number of accomplishments briefly cited were the completion of part I of Type Reactions in Fermentation Chemistry, a draft copy of which was passed around; the E. R. Squibb "Lectures on Chemistry of Microbial Products," delivered by Dr. Stodola, and soon to be published in book form; and the development of a process for making β -carotene by fermentation.

The organization and plans for handling Public Law 480 funds which have been made available for utilization research by contract or grant in foreign countries were described. Dr. Hilbert is heading up this undertaking. A review panel composed of the Associate Directors of the four Utilization Divisions will assist him in the screening and selection of research projects to be implemented under the program.

In conclusion Dr. Maclay stated that there had been a sharp increase in interest in our work on the part of industrial organizations, particularly the larger chemical corporations, during the past year. More than 1,400 visitors seeking technical information called on the laboratory during the past year as compared with about one-half that number in the previous year.

IV. RESEARCH RECOMMENDATIONS OF THE TECHNICAL ADVISORY COMMITTEE

Dr. J. W. Evans, who presided at this session, called upon Mr. G. T. Peckham and Drs. Scallet, Caldwell, Newton, Elder, Wilson, Gresham, and Goodwin to present some of the problems on which the Industry feels there is a decided need for more research.

The following technical problems were presented:

1. Need for a simpler, less costly, and time-consuming method than X-rays and flotation methods for determination of insect infestation in corn. An evaluation of the utility of infrared radiation in this regard was proposed in light of a patent (U. S. 2,820,147) recently issued on the use of I.R. to detect infestation in grain "such as wheat."
2. Development of a simple accurate method for determining heat damage in starch. The Congo red test and swollen granule method in current usage are either too slow or unreliable to adequately meet present needs.
3. Determination of the basic underlying principles which cause adhesiveness and tack in starch and dextrin pastes and the development of more precise methods for determining adhesiveness. Means of differentiating between adhesive and cohesive forces are also needed.
4. Determination of the causes of gel strength in starch. The degree of swelling and dispersion of starch granules needs to be related to the method of cooking, and a method for determining paste viscosities developed which will be universally accepted by the industry thus making effective communication possible. More information is needed on the behavior of starch pastes under high rates of shear as these conditions are encountered in most applications and very little data of this nature are available.
5. Development of methods to determine structural differences in pyrodextrins that would lead to a better understanding of the underlying causes for the different properties of white dextrins, canary dextrins, and British gums.
6. The development of a gravimetric chromatographic analysis method for carbohydrate hydrolyzates. The thought here is to eliminate colorimetric procedures by eluting, drying, and weighing the fractions and correlating results with those of the anthrone or phenol methods. Such a method, it was felt, would permit usable quantities of fractions, about which little is currently known, to be obtained.
7. The need to bring together and embody in one article all available information on starch structure.

8. An evaluation of the utility of infrared spectroscopy as an analytical tool for determining more about the structure of starch and starch derivatives.

In addition to these specific problems of immediate interest to the Industry, several broad concepts of research needs were presented as follows:

Dr. Gresham challenged research, "to learn something that no one knows about corn and starch." Asking for a major "break-through" on uses of starch, he raised the question of how to do basic research without becoming "bogged down" in industrial applications. He stated that in his opinion continued work in applying starch in its usual realms of use will not produce returns as great as exploratory research in completely new fields with the possibility of leading to a "break-through."

Dr. Scallet raised the question of whether starch is the primary product of corn. He asked whether screening and genetic work to improve corn's nutritional value, especially amino acid content, has been considered. Dr. Elder pointed out that this is a long-range concept and urged that research be undertaken with the geneticists now in order to have the necessary products at the time they will be needed, 10 to 15 years from now.

Dr. Elder urged more research on enzymic conversion of corn to industrial products, pointing out that it offers possibilities for new and improved competitive products. He commended the work that has gone into NU's forthcoming publication on "Type Reactions in Fermentation Chemistry" and stated that this is just what the Industry needs.

Dr. Newton pointed out that the problem of starch utilization was not only one of how to expand markets but how to retain present markets.

Dr. Goodwin stated that he believes our high-amylose program may be primarily a protective program and a much needed one.

Discussion

Everyone felt that this was a very worthwhile list of research proposals and a lively discussion as to how a number of these problems might best be attacked ensued. Dr. Maclay pointed out that under the present setup the grain infestation problem should be investigated by the Marketing Service rather than Utilization. Dr. Senti concurred in this and stated that the marketing group had already worked out what was purported to be an improved method for detection of infestation. In principle the method involves crushing a number of kernels against a paper strip sensitized with ninhydrin. Where infested kernels are encountered a colored spot develops on the paper. It was not known whether or not the insect had to be in a juicy condition to show up in the test.

In connection with the proposed use of I.R. spectroscopy in starch structure studies, Dr. Senti pointed out some studies along these lines had already been tried and that the problem was one of preparing starch in

suitable form for I.R. study without changing its structure in the process. The granule is too thick to be penetrated by I.R. and studies are currently being carried out on starch films prepared in various manners to determine whether any useful information can be obtained by this technique.

Dr. Maclay and Mr. Rist pointed out that considerable thought had been given to a program of developing corn with better protein composition but that the tremendous analytical task associated with such a problem had discouraged its initiation. Someone suggested that the Experiment Stations might help out with the analytical routine; however, Mr. Rist replied that they did not want to be service groups as Ph.D.s could not be trained on this basis. Mr. Peckham suggested that some of the analytical work might be circumvented by breeding for a larger germ since the germ is known to contain most of the worthwhile protein. Measurement of germ-size which is a relatively simple operation would then serve as a preliminary screening tool. Dr. Senti suggested that as a starter some of the exotic samples provided by high-amylose breeders might be examined. Dr. Elder urged that the problem be taken on now as it is a long-term proposition and that if we are to have the results when we need them we must start now. Dr. Maclay stated that we were very glad to have this support from the Industry and that the problem would be given further consideration.

V. PROGRESS REPORTS ON SELECTED PROJECTS AT THE NORTHERN UTILIZATION
RESEARCH AND DEVELOPMENT DIVISION AND
DISCUSSIONS ON THESE REPORTS

- A. Report of Work Supported by Corn Industries Research Foundation.-
Effect of Granule Pretreatment on the Intrinsic Viscosity of Amylose
in Aqueous Extracts of Corn and Wheat Starches E. M. Montgomery
and F. R. Senti

Investigation of amylose in centrifugal supernatants of aqueous dispersions of corn and wheat starches showed that the intrinsic viscosity of the linear component in alkali and in water is strongly affected by the preparative procedure. The starches were separated by a wet-milling procedure after steeping the grain at 37° C. in distilled water. Amylose of high purity was extracted in yields of 75-80 percent in concentrations of about 0.4 percent from 2 percent starch dispersions after the granular starch was pretreated in hot aqueous-glycerol or 2-ethoxyethanol mixtures. More than a twofold variation in the intrinsic viscosity of amylose in alkali or in water resulted from variation of the water content and temperature of the pretreatment solvent mixture. Extracted amylose of low intrinsic viscosity could be converted to high intrinsic viscosity by heating in saturated *n*-butanol solutions at 60° C. for 2 hours. Amylose of high intrinsic viscosity changed to low intrinsic viscosity on standing in solution. Solutions of amylose of high and low intrinsic viscosity differed markedly in their stability toward retrogradation. Interpretations of these results are given.

Discussion

Questions were raised as to whether these phenomena might be important in the retrogradation of whole starch, how can the differences in solubility of the various amyloses in Cupra B be accounted for, and whether antioxidants might be effective in stabilizing the high and low viscosity forms and furnish some insight into the mechanism of retrogradation.

Although amylopectin could have a stabilizing influence on amylose in whole starch, it was generally believed that the phenomenon observed with pure amylose might be related to the retrogradation of whole starch and that this phase of the problem should be looked into. Since antioxidants are strong hydrogen-bonding agents as well as oxygen acceptors, it was doubted that they could be used to advantage in elucidating the mechanism of amylose degradation as it would be difficult to decide which mechanism was operative in any stabilizing effects that might be observed.

- B. Nonprotein Nitrogenous Constituents of Corn J. S. Wall

Minor components of the cereal grains such as the nonprotein nitrogenous constituents, while probably not of direct economic importance, may affect the value of the grains in three ways: (1) Nutritionally, not only in human diet and animal feeds, but also as part of steep liquor or grain

supplements to fermentation media; (2) by contributions to flavor, odor, and color which influence commercial acceptance; (3) as factors facilitating or retarding germination, important in malting and seed viability. Studies were, therefore, undertaken at NU to determine the nature and quantities of the free nitrogen compounds present in extracts of corn.

While excellent methods were available for the chromatographic separation and determination of amino acids, purines and other compounds, comparable techniques were not available for investigating the quaternary nitrogen compound such as choline, trigonelline, and thiamine which constitute an appreciable fraction of the nitrogen of dialyzable portions of corn extracts. A sensitive method enabling the determination of microgram quantities of betaines and other quaternary amines was therefore developed. By reaction with iodine in KI solution the quaternary nitrogen compounds are converted to characteristic water-insoluble crystalline periodide complexes. Chilling and acidification of the reaction mixture was found to promote more quantitative precipitation of certain of the periodides. The isolated periodides upon solution in ethylene dichloride exhibit absorption maxima at 365 mμ in the ultraviolet. Standard curves relating optical density of the periodide solution and the concentration of the original quaternary nitrogen compounds have been prepared.

Employing the periodide procedure, it was possible to follow the separation of quaternary nitrogen compounds during chromatography on Dowex 50, ion-exchange resin, in the hydrogen form. Excellent resolution of these compounds was obtained by employing increasing concentrations of hydrochloric acid solutions as eluent. It was also possible to resolve and determine simultaneously amino acids, amides, purines, and a number of other types of nitrogenous substances by this system.

Concentrated extracts of corn containing the nonprotein nitrogen were prepared by macerating the corn with 80 percent ethanol and clarifying by centrifugation. Portions of extract were applied directly to the Dowex 50 column and subjected to chromatography. Some 25 individual well-characterized components were thus isolated and determined. The nonprotein nitrogen fraction constituted only about 1 percent of the total nitrogen of the corn (15 mg. N per 100 g. corn). Among the major components of this extract were asparagine, choline, alanine, proline, and γ-amino butyric acid, adenine, glutamic acid, arginine, and trigonelline.

Discussion

Dr. Wall, on being asked how the extracts were obtained, replied that they were obtained by either extraction with 80 percent ethanol or by absorption dialysis.

C. Composition of Cementing Layer Between the Germ and Endosperm in Corn M. J. Wolf

Studies were made of the pentosan content of the cementing layer and of cell walls in germ and endosperm adjacent to the cementing layer.

Pentosan content ranged from 68-95 percent. The pentosans were hydrolyzed from the isolated materials with hot 2 percent sulfuric acid. Arabinose and xylose were the principal constituents of the pentosans; smaller amounts of glucose and galactose were found in some regions.

Pentosanase activity was found in the dormant corn kernel and increased greatly as the kernel germinated. Optimum activity was obtained in corn germinated at 32° C. Treatment of the dormant and germinating kernel with pH 9.0 buffer solution favored maximum pentosanase activity. Ease of germ separation was correlated with pentosanase activity.

Discussion

Dr. Elder asked whether there might be a possibility of finding an enzyme that would effect a 100-percent release of starch from fiber and other constituents in corn economically. Dr. Wolf replied that such an investigation would be worthwhile.

D. Effect of Drying Conditions on the Suitability of Artificially Dried Corn for Starch Production M. M. MacMasters

The Northern Division has just concluded a study of artificially dried corn in cooperation with the Illinois Agricultural Experiment Station. The Station grew corn of known variety and harvested and dried it under known, controlled conditions. Workers at the Northern Division made laboratory studies on the processing characteristics of the dried corn.

Corn from 5 crop years (1952-1956) was harvested at approximately 30 percent and 20 percent moisture content, and dried at 6 air temperatures ranging between 120° F. (48.9° C.) and 200° F. (93.3° C.), each with air at 15 percent R.H. and 40 percent R.H., respectively. Drying conditions were such that the corn doubtless reached the temperature of the drying air. Controls were dried in ambient air.

Starch was separated under comparable, controlled conditions. Viability of each sample was determined by the 2,3,5-triphenyltetrazolium chloride color method. Processing data obtained included starch recovery, protein in starch, starch in gluten, protein in gluten, starch in fiber, and protein in fiber. Degermination was not attempted; germ was roughly separated by hand on the screen.

Statistical analysis showed that corn which had reached a temperature of 180° F. (82.2° C.), or over, gave poor starch recovery and high protein content in the recovered starch. Both starch recovery and protein content of the starch were satisfactory if the corn had attained a temperature no higher than 130° F. (54.6° C.). Samples of corn which had reached temperatures above 130° F. but below 180° F. gave variable results.

Viability was always decreased when the grain reached a temperature above 140° F. (60° C.). Since good degermination and high quality of the recovered corn oil are essential to the economy of wet milling, it appears that corn which has reached a temperature above 140° F. during drying is unsuited for use in starch production.

Discussion

Questions were raised as to what temperatures are recommended by manufacturers of drying equipment, and how does the cost of drying at 140° C. compare with that at higher temperatures.

No one seemed to have a definite answer to the first question; however, it was mentioned that some dryers are built to reach temperatures as high as 250° F. Dr. MacMasters stated that comparative data on the cost of drying at different temperatures were not available but that the time element and handling practices rather than fuel costs were most important.

E. Dialdehyde Starch Production -- Demonstration of Electrolytic Cells in Pilot Plant V. E. Sohns

The visitors were shown the operation and pertinent construction details of a series of electrolytic cells, each having a capacity for the oxidation of 15 pounds of corn starch per run under the conditions outlined by Mehlretter, et al. in "Electrolytic Preparation of Periodate Oxystarch," Ind. Eng. Chem. 49, 350 (1957).

The essential features of each cell are: Six anodes, connected in parallel, of 1/8-inch thick, 1 percent silver lead alloy having a submerged depth of 19 cm., and a width of 12.5 cm.

Fourteen cathodes, connected in parallel, of 5/16-inch iron rod suspended inside cathode diaphragms of alundum RA 84 filtering thimbles 38 mm. in diameter and 270 mm. long which are suspended in pairs on each side of each anode.

The total anode area is approximately 2,850 sq. cm. so that at a current of 80 amps. the resulting current density is 0.028 amps./cm.² of submerged anode surface. The entire assembly is mounted in a lead-lined box provided with a cooling coil in the bottom. Two laboratory-size stirrers are mounted in each cell to provide agitation.

In these cells 15 pounds of starch (dry basis) can be oxidized to approximately 90 percent dialdehyde content in a matter of about 40 hours using a mole ratio of iodate to starch of 1 to 10 and a current density of 0.028 amp./cm.² while operating at a temperature of 25° C. or slightly below. The product is readily recovered and freed of the oxidizing medium by filtration or centrifugation followed by washing twice with water. The first water-wash removes the bulk of the oxidizing medium adhering to the product and is retained for reuse in subsequent oxidations following concentration by evaporative removal of water. The second wash contains about 6 percent of the original charge of oxidant but is too dilute to make recovery economical. It was pointed out that fresh oxidant was currently being used in each run but that studies on recycling of the oxidant were soon to be initiated to determine whether results comparable to those reported for laboratory-scale preparations could be achieved.

Discussion

Mr. Sohns was asked how many recycles had been carried out in the laboratory without encountering difficulty and what losses were encountered on cleaning up and reconstituting the oxidant. Mr. Sohns replied that as many as four consecutive oxidations with the same charge of oxidant had been carried out in the laboratory without encountering difficulty and that about a 12-percent loss was encountered in preliminary studies on the purification of used oxidant.

F. Fractionation of Corn Protein E. M. Craine

Proteins extracted from corn meal with water, salt solutions, and carbonate buffers were examined electrophoretically. Of the components detected, four were partially extracted with water but more completely extracted with salt solutions and carbonate buffer. In addition to these four components, two more were extracted with saline, while carbonate buffer at pH 9, in addition to the components extracted with saline, removed still another component.

Most of the additional protein extracted with carbonate buffers consisted of one electrophoretic component. In purified solution where the phytate ions had been removed, the protein exhibited an isoelectric point in the regions of pH 6 to 7. Thus, it was typical of a globulin rather than the classical glutelins. Heretofore in classification systems the globulins of corn had been limited to those extracted with saline.

Lowering the pH of aqueous extracts of corn induced precipitation of protein containing considerable phosphorus. These precipitates did not occur when phytate ions were removed. However, the precipitates were again obtained when the phytate ions were replaced. Most of the phosphorus in corn is in phytate form and was found to occur in aqueous extracts. These observations together with analytical data on the composition of various extracts and fractions therefrom, plus the reports of other investigators on the formation of insoluble combinations of protein and phytate at low pH values, indicate that the formation of complexes between corn globulins and phytate takes place.

It is proposed that in the presence of protein and phytate ions the solubility of protein-phytate complexes is involved rather than the solubility of proteins. In keeping with this concept the following system of equilibriums is visualized as being operative.



Three factors influence these equilibriums: Ionic strength, pH, and concentration of phytate. An increased ionic strength or pH shifts the equilibriums toward the left giving uncombined protein and phytate ions.

No evidence has been obtained to show the influence of phytate concentration but according to the Law of Mass Action an increase in phytate concentration should enhance complex formation.

All of the observations made on the behavior of the protein in water, saline, or carbonate buffer extracts of corn fit into this working theory. These data and concepts support the contention that globulins are quantitatively extracted at pH 9 with carbonate buffer, and that this method in conjunction with electrophoretic assay and other measurements may be used to advantage in discerning variations in protein composition in different types and varieties of corn.

Discussion

Dr. A. L. Wilson, Assistant Director of Research, Corn Products Refining Company (Argo, Illinois), suggested that information more useful to the industry might be obtained if this work were carried out on industrial fractions of corn rather than on corn meal or whole corn. Dr. Craine said that this might well be and that work in these areas was planned.

G. Microbial Production of β -Carotene Alex Ciegler

Beta-carotene, which is converted in the body to vitamin A, finds large uses in mixed feeds and fortified foods to provide both vitamin content and yellow color.

Although it has been known for many years that certain microorganisms produce β -carotene, the quantities were too small to make its production by fermentation attractive from the commercial standpoint. The potentialities of fermentation methods for this type of synthesis, however, were heightened considerably by the recent disclosure of Barnett, Lilly, and Krause (Science 123, 141 (1956)) that mated strains of Choanephora cucurbitarum produced much higher yields of β -carotene than those obtained with the same organisms cultured separately, i.e., unmated. Following this disclosure numerous mating types of members of the family Choanephoraceae available in our culture collection were screened for their ability to produce β -carotene in a synthetic medium composed of inorganic salts, thiamine, dextrose, and asparagine. Two strains of Blakeslea trispora, NRRL 2456 (+ mating type) and NRRL 2457 (- mating type) were found which, when paired, gave considerably higher yields of β -carotene than had been reported previously.

Subsequently, it was shown that the organic carbon and nitrogen sources in the synthetic medium could be replaced by a number of cereal grains and grain products, thus effecting considerable savings in cost. Maximum yields were obtained using soybean meal. Results, however, were not too reproducible. The addition of corn to the soybean meal gave both reproducibility and high yields. The polyene and β -ionone structures present in β -carotene led to the supposition that the incorporation of β -ionone and unsaturated fats in the medium might improve yields. This supposition

was borne out experimentally in that each of these materials significantly stimulated the production of β -carotene. Fats containing primarily oleic and linoleic acids were particularly effective in this regard. Synthesis of carotene was also favorably influenced by the addition of surface active agents which served to emulsify the fats. By incorporation all of the above factors into a single medium a process was evolved which gave yields of β -carotene as high as 0.75 percent of the total dried solids produced. Preliminary cost calculations indicate that on this basis β -carotene can be produced in the crude form at a cost of \$100 per kilogram as compared with a present cost of \$290 per kilogram for the commercial synthetic product. Feeding tests with rats and chicks have shown that the product is a good source of vitamin A for rats and preliminary results with chicks are encouraging.

Discussion

None.

H. Cooperative Program on the Development of High-Amylose Corn F. R. Senti

Milestones in the development of high-amylose corn since the early 1940's when the Northern Division first became interested in this program were reviewed. The fact that corn was at that time known which had starch that was all amylopectin suggested that it might be possible to develop a corn with starch that was all amylose. Early in 1945, therefore, a search was initiated at NU for a natural source of high-amylose starch. Hundreds of grain samples and other plant sources of starch were analyzed in this preliminary screening program. The most immediate result of this search was the discovery of high-amylose starch in peas. Although the starch in various varieties of wrinkled-seeded peas was found to contain as much as 60-70 percent amylose, peas were not an attractive source of amylose as they contained only about 45 percent starch. Further attention was then directed to cereal grains and in 1949 a corn supplied by the Bear Hybrid Corn Company was found to contain starch with a 36-percent amylose content. Meanwhile, Cameron, in 1947, working at Harvard with analyses supplied by Iowa State College, studied the effect of different genetic combinations on the carbohydrate content of corn and found that certain combinations of genes could give corn with starch of 65 percent amylose content, but in the corn studied the total starch content was very low. The corn with starch of 36-percent amylose content examined at NU, on the other hand, appeared to be like field corn in its total starch content.

In 1950, the Northern Division expanded its cooperative efforts with plant breeders to determine if breeding might produce the desired type of corn. In cooperation with Crops Production, ARS, USDA (represented by Dr. Zuber who cooperates with the Missouri Agricultural Experiment Station) and the Bear Hybrid Corn Company over a thousand samples were examined annually for amylose content over the next several years. Inbred lines, foreign varieties, and crosses produced by breeders were included in this study.

In 1952, Mr. Robert P. Bear of the Bear Hybrid Corn Company reported a gene (ae) which increased the amylose content of corn in which it appeared. Working with this gene a corn was developed by 1954 which contained 60 percent starch of which 62 percent was amylose. This was the first high-amylose corn of adequate starch content to be of industrial interest. Kramer in 1956 confirmed Bear's new (ae) gene and the results reported earlier by Bear and NU, viz., over 60 percent amylose content without much starch deficiency.

In 1956 NU greatly increased its cooperative work with both Dr. Zuber and Mr. Bear. From December 1956 to October 1957 about 8,000 samples were analyzed for amylose content of the starch. During the period from October 1957 to October 1958 the analysis of 13,000 additional samples is anticipated. This intensified analytical program has permitted the breeders to weed out unpromising material much earlier than before with the result that more rapid progress was made and samples with starch of over 70-percent amylose content are becoming fairly common. One sample supplied by Dr. Zuber in 1957 contained starch which analyzed for over 80 percent amylose content and although only one such sample has been obtained to date cooperating breeders are confident of attaining even higher amylose content.

Preliminary studies of one corn with 60 percent starch, of which 56 percent was amylose, by iodometric analysis have shown that this corn presents some processing difficulties not ordinarily encountered. The starch granules were smaller than normal and irregular in shape. The protein and starch were somewhat more difficult to separate from each other. The starch which was obtained by the normal wet-milling procedure contained considerably more protein than is present in a good quality commercial corn starch. Further processing studies are in progress to develop ways to obtain a higher quality starch from high-amylose corn. Starch from this sample of corn was fractionated by two laboratory procedures. After three recrystallizations, 43 percent of pure amylose was obtained. The discrepancy between the amylose content actually recovered and that found by iodometric titration was later found to be due to the fact that the amylopectin in this starch, contrary to its normal behavior, had an appreciable affinity for iodine. Further studies with this amylopectin showed that it was more difficultly soluble than the ordinary variety and appeared to be more linear in character. Moreover, the branches in this amylopectin appeared to be longer than usual. This no doubt accounts for its affinity for iodine. The amylose fraction from this starch was found to be like that of ordinary corn starch in molecular structure and size.

More recently one-pound samples of several corns with starch up to 75 percent amylose content have been received and such studies as are permitted with samples of this size are being undertaken. These include methods of dispersion, viscosity determinations on pastes and solutions, etc.

Discussion

Questions were raised as to what methods of dispersion were employed in the preparation of samples for films, whether acetates of high-amylose starch had been prepared and whether the branches in amylopectin were likely to get longer as the amylose content of the starch increased.

Dr. Senti replied that the use of formamide, alkaline pretreatment both with and without subsequent butanol treatment, as well as autoclaving, had been used to effect dispersion. No acetates had been prepared. Present evidence suggests that length of the amylopectin branches will increase as starches of higher amylose content are developed.

VI. CLOSING REMARKS

Dr. Maclay thanked all those who had taken part in the program and again expressed his appreciation to members of the CIRF for the opportunity to acquaint them with the program of the Laboratory as well as for the part they had taken in presenting a number of worthy research proposals.

Dr. Goodwin, speaking on behalf of the CIRF members, stated that he felt this had been a very profitable meeting and commended the Laboratory for the excellent program that was presented. He regretted that time would not permit taking up a number of specific proposals on the industrial use of grains set forth in the Corn Wet Milling Industries Report to the President's Commission as originally planned but stated that these could be discussed at a later date if desired.

